Human Recombinant Gqi5 Stable Cell Line

Technical Manual No. TM0571 **Version 10132010**

I. Introduction

Catalog Number: M00455

Cell Line Name: CHO-K1/Gqi5

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Application: Functional assay for Gi/o-coupled GPCR receptors

Freeze Medium: 45% complete growth medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

1. CHO-K1/Gqi5

CHO-K1/Gqi5 is a CHO-K1 cell line stably expressing the chimeric Gqi5 alpha subunit protein which a chimeric Gq protein instead of the last five carboxyl-terminal amino acids from Gi. It is used as a host cell for transfection expression of Gi/o-coupled receptors, the constitutively expressed Gqi5 protein in the cells allows many transfected receptors which normally inhibit the cAMP pathway, to couple to Gq signal transduction and mobilize intracellular calcium. The cell line carries the hygromycin B resistance gene and is resistant to hygromycin B.

2. The sequence of Gqi5

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atggcccgctcgctgacctggcgctgctgcccctggtgcctgacggaggatgagaaggccgccgcccgggtggaccagga
gatcaacaggatcctcttggagcagaagaagcaggaccgcggggagctgaagctgctgcttttgggcccaggcgagagcg
ggaagagcaccttcatcaagcagatgcggatcatccacggcgccggctactcggaggaggagcgcaagggcttccggccc
ctggtctaccagaacatcttcgtgtccatgcgggccatgatcgaggccatggagcggctgcagattccattcagcaggcc
cgagagcaagcaccacgctagcctggtcatgagccaggacccctataaagtgaccacgtttgagaagcgctacgctgcgg
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gtgtactacctgtcccacctggagcgcatcaccgaggagggctacgtccccacagctcaggacgtgctccgcagccgcat
gcccaccactggcatcaacgagtactgcttctccgtgcagaaaaccaacctgcggatcgtggacgtcgggggccagaagt
cagagcgtaagaaatggatccattgtttcgagaacgtgatcgccctcatctacctggcctcactgagtgaatacgaccag
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§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

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tgcctggaggagaacaaccaggagaaccgcatgaaggagagcctcgcattgtttgggactatcctggaactaccctggtt
caaaagcacatccgtcatcctctttctcaacaaaaccgacatcctggaggagaaaatccccacctcccacctggctacct
atttccccagtttccagggccctaagcaggatgctgaggcagccaagaggttcatcctggacatgtacacgaggatgtac
accgggtgcgtggacggccccgagggcagcaagaagggcgcacgatcccgacgcctcttcagccactacacatgtgccac
agacacacagaacatccgcaaggtcttcaaggacgtgcgggactcggtgctcgcccgctacctggacgag**TGTGGCCTCT**
TCTGA

III. Thawing and Subculturing

Thawing protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B to concentrations of 100 µg/ml Hygromycin B in the following day.

Subculturing protocol

1. Remove and discard culture medium.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution to 10 cm dish and observe the cells under an inverted microscope until cell layer is detached (usually within 3 to 5 minutes).
   Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate detaching.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in the culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

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